IN THE SPECIFICATION

Please replace the Title with the following:
"Novel Carotenoid ε- and β-Hydroxylases for use in engineering carotenoid metabolism in plants."

Please replace paragraph [0156] with the following:
[0156] The *LUT1* locus has previously been mapped to the bottom arm of chromosome 3 at 67 ± 3 cM (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001), herein incorporated by reference). For fine mapping of the locus, 530 plants homozygous for the *lut1* mutation were identified from approximately 2,000 plants in a segregating F_2 mapping population. Using SSLP markers, *LUT1* was initially localized to an interval spanning two BAC clones (F8J2 and T4D2) and was further delineated to a 100 kb interval containing 30 predicted proteins (Fig. 2*A*). The term "BAC" and "bacterial artificial chromosome" refers to a vector carrying a genomic DNA insert, typically 100-200 kb. The term "SSLP" and "simple sequence length polymorphisms" refers to a unit sequence of DNA (2 to 4 bp) that is repeated multiple times in tandem wherein common examples of these in mammalian genomes include runs of dinucleotide or trinucleotide repeats (for example, CACACACACACACACACA (SEQ ID NO:59)." As with all other carotenoid biosynthetic enzymes, the *LUT1* gene product is predicted to be chloroplast-targeted and within the 100 kb interval containing *LUT1*, six proteins were predicted as being chloroplast-targeted by the TargetP prediction software (Emanuelsson et al., (2000) J. Mol. Biol., 300: 1005-1016 and Henrik et al., (1997) Protein Engineering, 10:1-6).~~—www.~~ on the world wide web at cbs.dtu.dk/services/TargetP). One of these chloroplast-targeted proteins, At3g53130, is a member of the cytochrome P450 monooxygenase family (CYP97C1). Cytochrome P450 monooxygenases are heme-binding proteins that insert a single oxygen atom into substrates, e.g. hydroxylation reactions, and therefore At3g53130 was considered to be a strong candidate for *LUT1*.

Please replace paragraph [0162] with the following:

[0162] Genomic DNA from homozygous *lut1* F_2 plants was isolated using the DNAzol reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA). PCR reactions were performed with 1 μl of genomic DNA in a 20 μl reaction mixture. The PCR program was 94° C for 3 min, 60 cycles of 94° C for 15 s, 50° C-60° C (the annealing temperature was optimized for each specific pair of primers) for 30 s, 72° C for 30 s, and finally 72° C for 10 min. A portion of the PCR product was then separated on a 3% agarose gel. *lut1* had been previously mapped to 67 ± 3 cM on chromosome 3 (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001). Simple Sequence Length Polymorphism (SSLP) markers for fine mapping in this interval were designed based on the insertions/deletions (INDELs) information obtained from the Monsanto website:~~www~~. on the world wide web at arabidopsis.org/Cereon/.

Please replace paragraph [0169] with the following:

[0169] The chloroplast transit peptide prediction software ChloroP v 1.1 (~~www~~. on the world wide web at cbs.dtu.dk/services/ChloroP/) predicts an N-terminal transit peptide in LUT1 that is cleaved between Arg-36 and Ser-37 (Fig. 2*C*). The predicted chloroplast localization for LUT1 is consistent with the subcellular localization of carotenoid biosynthesis in higher plants (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998)) but is uncommon for a plant cytochrome P450. Out of the 272 predicted cytochrome P450s in the Arabidopsis genome, only nine, including LUT1, are predicted to be chloroplast-targeted (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). LUT1 also contains a single predicted transmembrane domain (shaded box, Fig. 2*C*), which contrasts with the four transmembrane domains predicted for the non-heme di-iron β-hydroxylases (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference). Initial attempts to express and assay LUT1 protein in yeast were unsuccessful.

Please replace paragraph [0174] with the following:

[0174] Our *Arabidopsis* LUT1 sequence was previously designated as CYP97C1 according to the standardized cytochrome P450 nomenclature (~~www~~. on the world wide

web at biobase.dk/P450). The *Arabidopsis* genome also contains two other CYP97 family members, CYP97A3 and CYP97B3, which are 49% and 42% identical to the LUT1 polypeptide, respectively. Interestingly, CYP97A3 (At1g31800) is also one of the nine cytochrome P450s in Arabidopsis predicted to be chloroplast-targeted, while CYP97B3 (At4g15110) is predicted to be targeted to the mitochondria (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). Additional CYP97 family proteins were identified in the EST and genomic databases from a wide variety of monocots and dicots, including *Arabidopsis*, barley, rice, wheat, soybean, pea, sunflower, tomato, and diatom (Figs. 5 and 8). The term "EST" and "expressed sequence tag" refers to a unique stretch of DNA within a coding region of a gene; approximately 200 to 600 base pairs in length. The term "contig" refers to an overlapping collection of sequences or clones.

Please replace paragraph [0326] with the following:

[0326] Genomic DNA from homozygous *lut1* F_2 plants was isolated using the DNAzol reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA). PCR reactions were performed with 1 μl of genomic DNA in a 20 μl reaction mixture. The PCR program was 94° C for 3 min, 60 cycles of 94° C for 15 s, 50° C-60° C (the annealing temperature was optimized for each specific pair of primers) for 30 s, 72° C for 30 s, and finally 72° C for 10 min. A portion of the PCR product was then separated on a 3% agarose gel. *lut1* had been previously mapped to 67 ± 3 cM on chromosome 3 (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001)). Additional Simple Sequence Length Polymorphism (SSLP) markers for fine mapping in this interval were designed based on the insertions/deletions (INDELs) information obtained from the Monsanto website: ~~www~~. on the world wide web at arabidopsis.org/Cereon/.

Please replace paragraph [0328] with the following:

[0328] Isolation of T-DNA Knockout Mutants in At3g53130 and Generation of a Carotenoid Hydroxylase Triple Knockout Mutant Line. At3g53130 specific primers (forward, 5'-CTTCCTCTTCTTACTCTTCTCTCTTCACT-3' (SEQ ID NO:28); reverse, 5'-AAGAACGATGGATGTTATAGACTGAAATC-3' (SEQ ID NO:29)) were sent to the

University of Wisconsin Arabidopsis T-DNA knockout facility to identify knockout mutants of the *LUT1* gene. A single knockout line, designated *lut1-3*, was identified and isolated as described (~~www~~. on the world wide web at biotech.wisc.edu/Arabidopsis/). In order to generate a hydroxylase triple knockout mutant line, homozygous *lut1-3* and *b1 b2* plants were crossed. Putative *lut1-3 b1 b2* triple mutants were identified from the segregating F_2 population by HPLC and their genotypes confirmed by PCR as previously described (Tian, *et al. Plant Cell* 15, 1320-1332 (2003), herein incorporated by reference).

Please replace paragraph [0334] with the following:

[0334] The *LUT1* locus has previously been mapped to the bottom arm of chromosome 3 at 67 ± 3 cM (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001), herein incorporated by reference). For fine mapping of the locus, 530 plants homozygous for the *lut1* mutation were identified from approximately 2,000 plants in a segregating F_2 mapping population. Using SSLP markers, *LUT1* was initially localized to an interval spanning two BAC clones (F8J2 and T4D2) and was further delineated to a 100 kb interval containing 30 predicted proteins (Fig. 2*A*). As with all other carotenoid biosynthetic enzymes, the *LUT1* gene product is predicted to be chloroplast-targeted and within the 100 kb interval containing *LUT1*, six proteins were predicted as being chloroplast-targeted by the TargetP prediction software (~~www~~. on the world wide web at cbs.dtu.dk/services/TargetP). One of these chloroplast-targeted proteins, At3g53130, is a member of the cytochrome P450 monooxygenase family (CYP97C1). Cytochrome P450 monooxygenases are heme-binding proteins that insert a single oxygen atom into substrates, *e.g.* hydroxylation reactions, and therefore At3g53130 was considered to be a strong candidate for *LUT1*.

Please replace paragraph [0339] with the following:

[0339] The chloroplast transit peptide prediction software ChloroP v 1.1 (~~www~~. on the world wide web at cbs.dtu.dk/services/ChloroP/) predicts an N-terminal transit peptide in LUT1 that is cleaved between Arg-36 and Ser-37 (Fig. 2C). The predicted chloroplast localization for LUT1 is consistent with the subcellular localization of carotenoid

biosynthesis in higher plants (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference) but is uncommon for a plant cytochrome P450. Out of the 272 predicted cytochrome P450s in the Arabidopsis genome, only nine, including LUT1, are predicted to be chloroplast-targeted (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). LUT1 also contains a single predicted transmembrane domain (shaded box, Fig. 2*C*), which contrasts with the four transmembrane domains predicted for the non-heme di-iron β-hydroxylases (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference). Initial attempts to express and assay LUT1 protein in yeast were unsuccessful.

Please replace paragraph [0342] with the following:

[0342] Arabidopsis LUT1 was previously designated as CYP97C1 according to the standardized cytochrome P450 nomenclature (~~www~~. on the world wide web at biobase.dk/P450). The Arabidopsis genome also contains two other CYP97 family members, CYP97A3 and CYP97B3, which are 49% and 42% identical to the LUT1 protein, respectively. Interestingly, CYP97A3 (At1g31800) is also one of the nine cytochrome P450s in Arabidopsis predicted to be chloroplast-targeted, while CYP97B3 (At4g15110) is predicted to be targeted to the mitochondria (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). Additional CYP97 family proteins were identified in the EST and genomic databases from a wide variety of monocots and dicots, including Arabidopsis, barley, rice, soybean, and pea (Fig. 5).